Supplemental Figures and Tables

Supplementary Table 1: CIP Grade definitions (CTCAE)

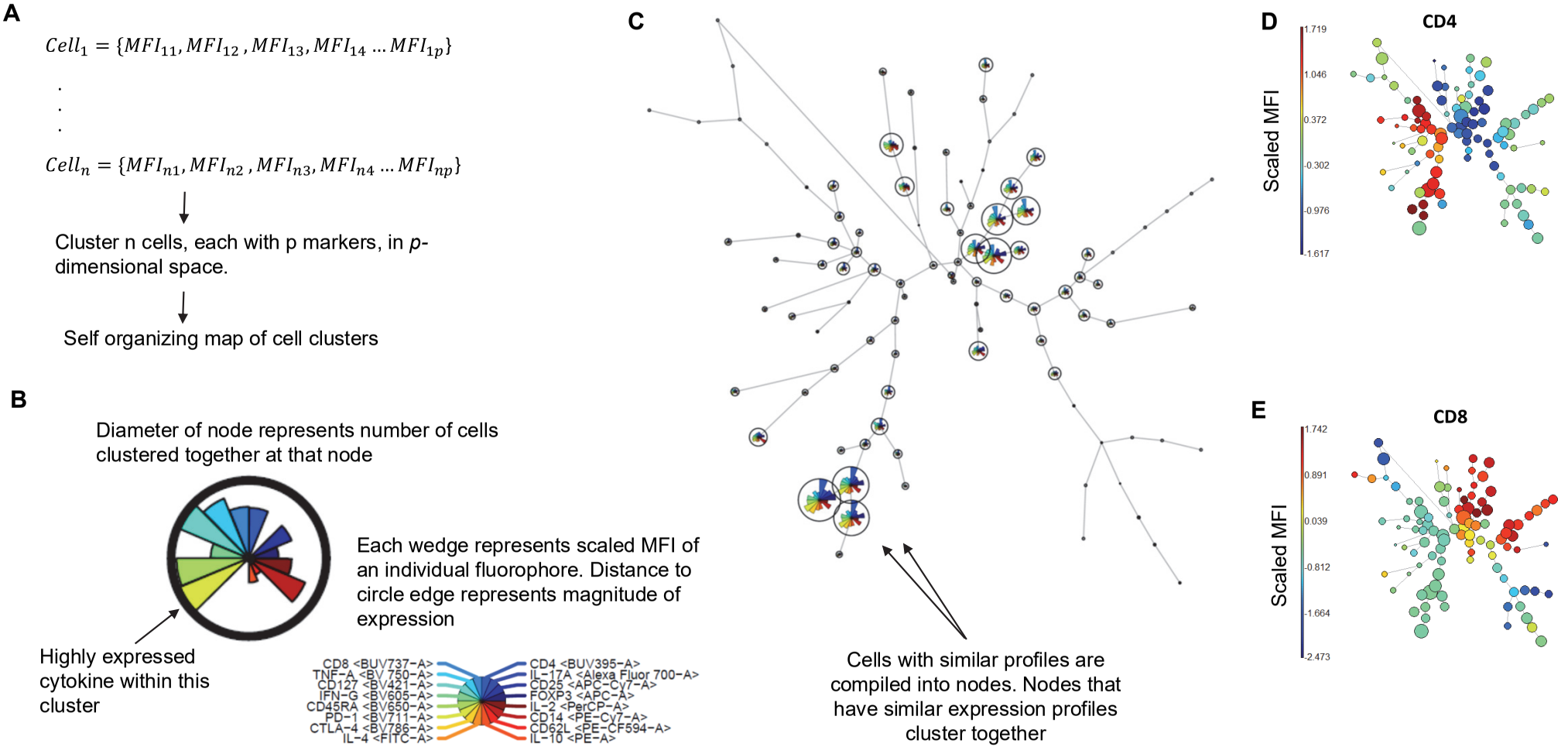
Grade	Pneumonitis
0	None
1	Presence of radiographic infiltrates, no symptoms
2	Symptomatic but no change in ability to perform ADL
3	Symptomatic, with significant dyspnea, desaturation with exertion
4	Hypoxemic respiratory failure
5	Death

ADL: Activities of daily living

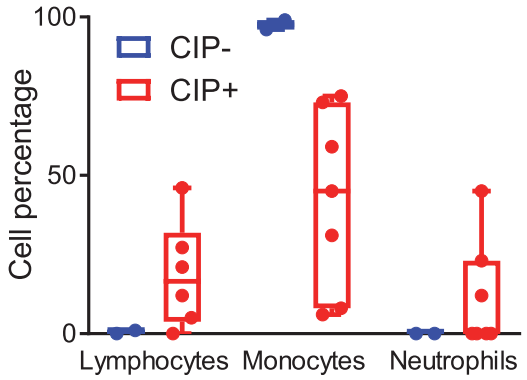
Supplementary Table 2: CIP Grade, Management and Outcomes

	n (%)
Pneumonitis Grade	
Grade 2	4 (33)
Grade 3	5 (41)
Grade 4	1 (8)
Grade 5	2 (16)
CIP Outcome (after steroid initiation)	
Resolved	6 (50)
Unresolved	6 (50)
Second Line-Agent	
None	4 (18)
IVIG	1 (9)
Infliximab	1 (9)

CIP: checkpoint inhibitor pneumonitis



Supplemental Figure 1: A) Algorithm for unsupervised clustering. Each cell is assumed to occupy a point in *n*-dimensional space, where n is the number of fluorophores, and the value in each dimension is the scaled, transformed MFI value for an individual fluorophore. A neural network algorithm is used to construct a self-organizing map that maximizes intra-cluster purity (similarity between cells within a cluster). B) The characteristics of each node are displayed in a star chart as shown. C) Cluster map for unstimulated control T cells. Cells subsets occupy distinct areas in the map; for instance, $CD4^{hi}$ cells occupy the left side of the T-cell map (D) while $CD8^{hi}$ cells occupy the right top portion (E).



Supplemental Figure 2: Manual cell differentials of lymphocytes, monocytes and neutrophils (per 500 total counted cells) in cell pellet stains of BALF specimens

Supplementary Table 3: Flow cytometry panels for myeloid and T-cells in BAL

Monocyte/B-cell panel

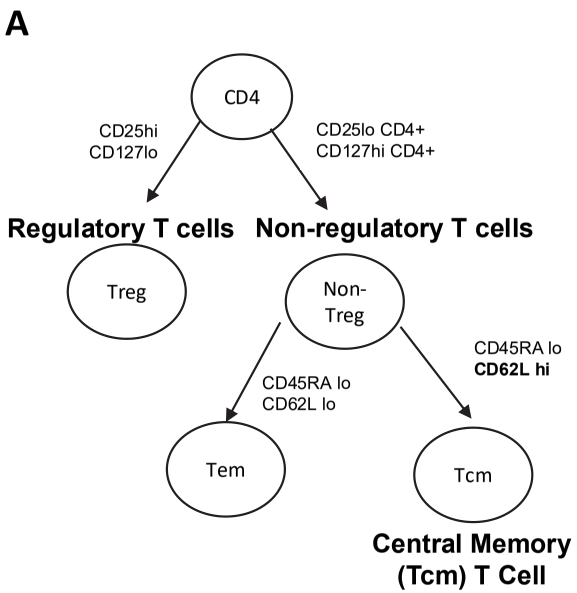
	ANTIBODY	FLOUROCHROME	CLONE	Company	Catalog #
Blue Laser 488nm	CD3	BB515	UCHT1	BD	564465
	IL-1RA	PE	AS-17	BD	340525
	CD19	BB700	SJ25C1	BD	566396
	CD80	PE-Cy7	L307	BD	561128
	TGF-b	PE-CF594	TW4-9E7	BD	562422
				BD	
Red Laser 635nm	IL-10	APC	JES3-19F1	BD	554707
	HLA-DR	APC-Cy7	G46-6	BD	561358
	PD-L1	APC-R700	MIH1	BD	565188
Violet Laser 405nm	TNF-a	BV605	Mab11	BD	563915
	IL-1beta	BV421	H1b-98	Biolegend	511710
	IL-8	BV510	G265-8	BD	563311
	CD11b	BV650	M1/70	BD	563402
	CD86	BV711	2331 (FUN-1)	BD	563158
	CD206	BV786	19.2	BD	740999
UV laser 355nm	Live/Dead	L/D		Invitrogen	L23105
	CD16	BUV395	3G8	BD	563785
	CD14	BUV737	M5E2	BD	564444

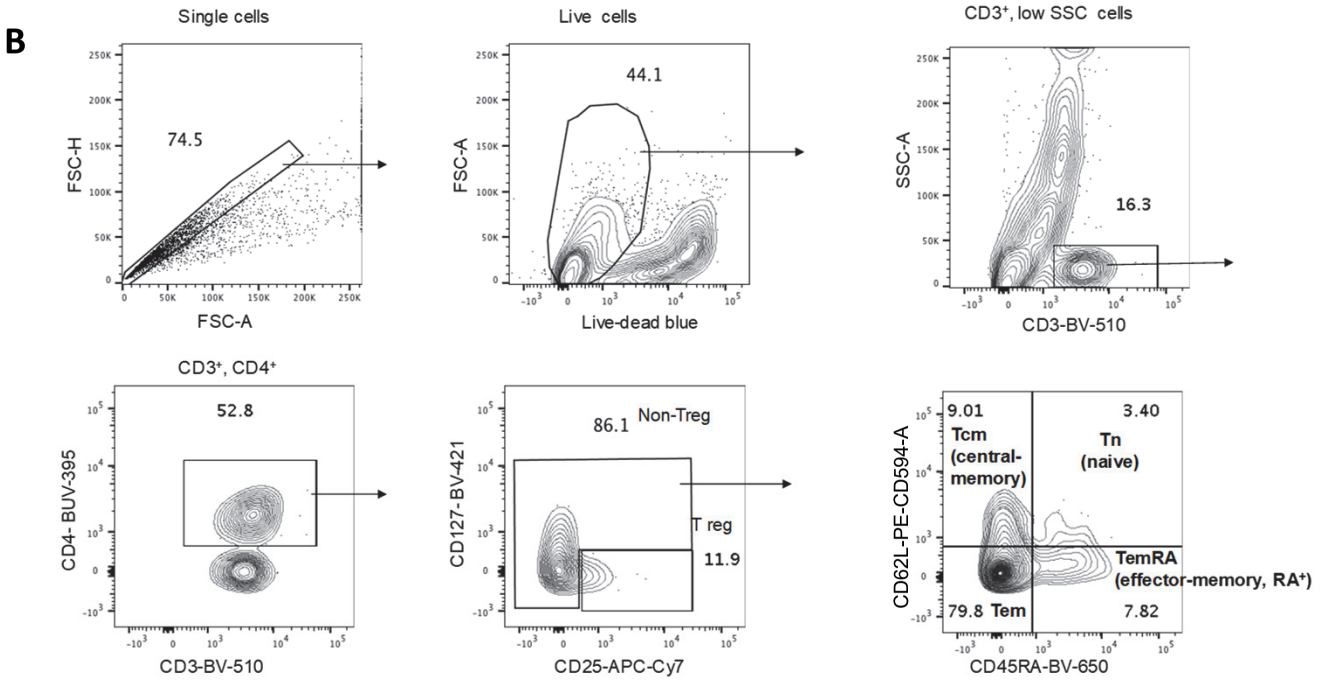
T-cell Panel

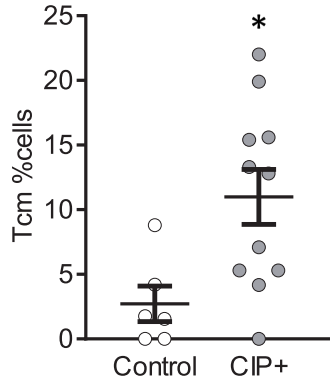
	ANTIBODY	FLOUROCHROME	CLONE	Company	Catalog #
Blue Laser 488nm	IL-4	Ax488	8D4-8	BD	557990
	IL-10	PE	JES3-9D7	BD	554498
	IL-2	BB700	MQ1-17H12	BD	566405
	CD14	PE-Cy7	MoP9	BD	562698
	CD62L	PE-CF594	DREG-56	BD	562301
Red Laser 635nm	Foxp3	APC	PCH101	Thermo Fischer	17-4776-42
	CD25	APC-C7	M-A251	BD	557753
	IL-17A	APC-R700	N49-653	BD	565163
Violet Laser 405nm	IFN-gamma	BV605	B27	BD	562974
	CD127	BV421	HIL-7R-M21	BD	562436
	CD3	BV510	UCHT1	BD	563109
	CD45RA	BV650	HI100	BD	563963
	PD-1	BV711	EH12	BD	564017
	TNF-a	BV750	MAb11	BD	566359
	CTLA-4	BV786	BNI3	BD	563931
UV laser 355nm	Live/Dead	L/D		Invitrogen	L23105
	CD4	BUV395	RPA-T4	BD	564724
	CD8	BUV737	SK1	BD	564629

Unstim			Stim		
# cells	Control	CIP		Control	CIP
T-cells	8296	27775	T-cells	5759	28685
Mono/B	50569	28442	Mono/B	76748	33034

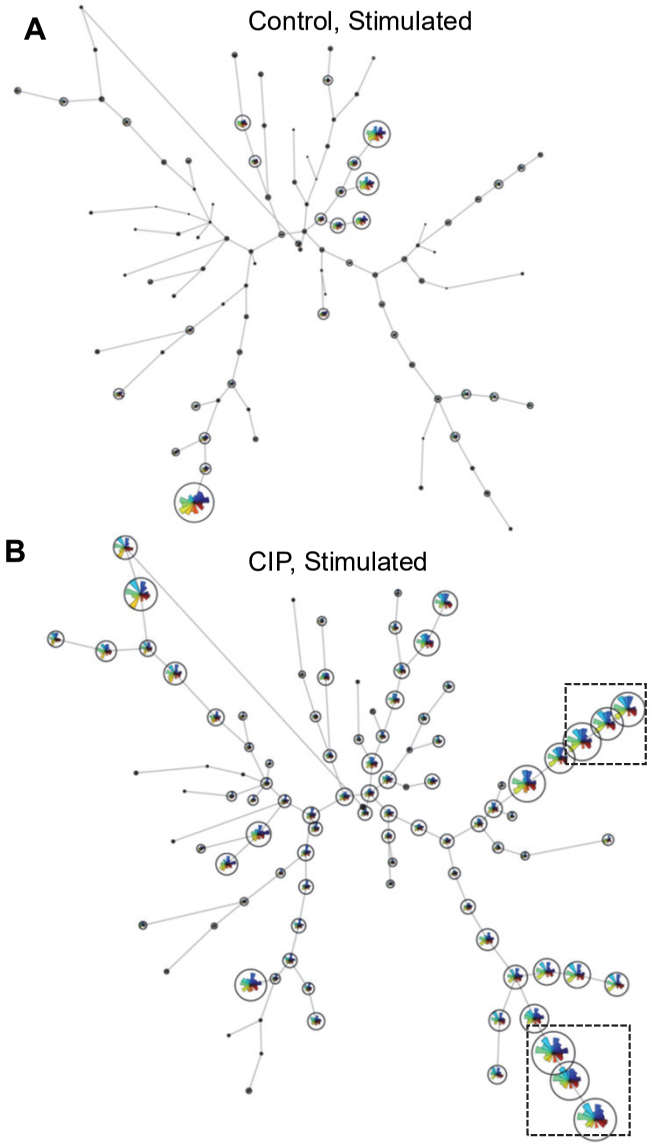
Supplemental Table 4: Number of cells analyzed under unstimulated and stimulated conditions.

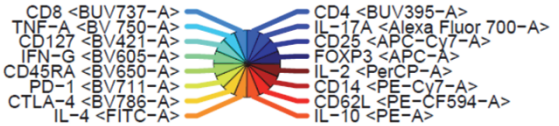




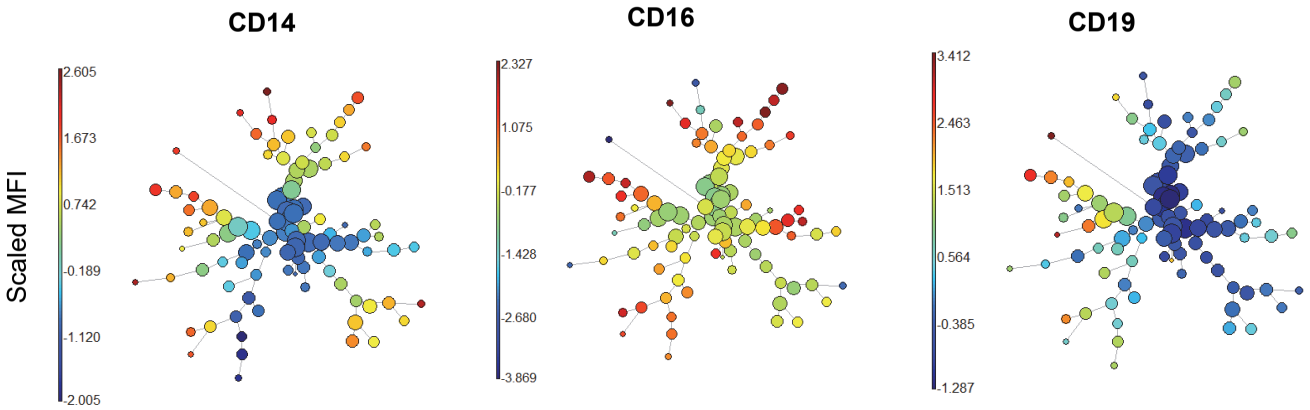


Supplemental Figure 3: A) Schematic showing CD4 subpopulations in human lymphocytes. B) Manual gating strategy for isolating Treg and Tcon subpopulations. C) Scatter plot showing increased Tcm in CIP compared to controls * $p < 0.05$

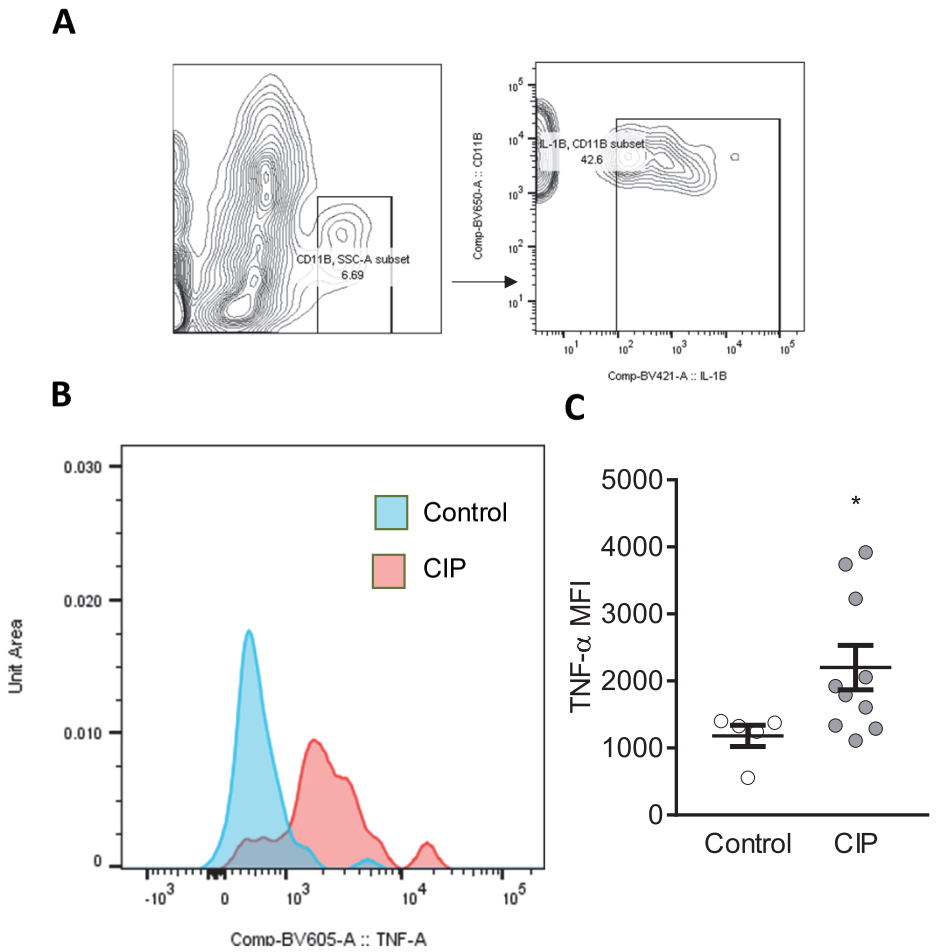




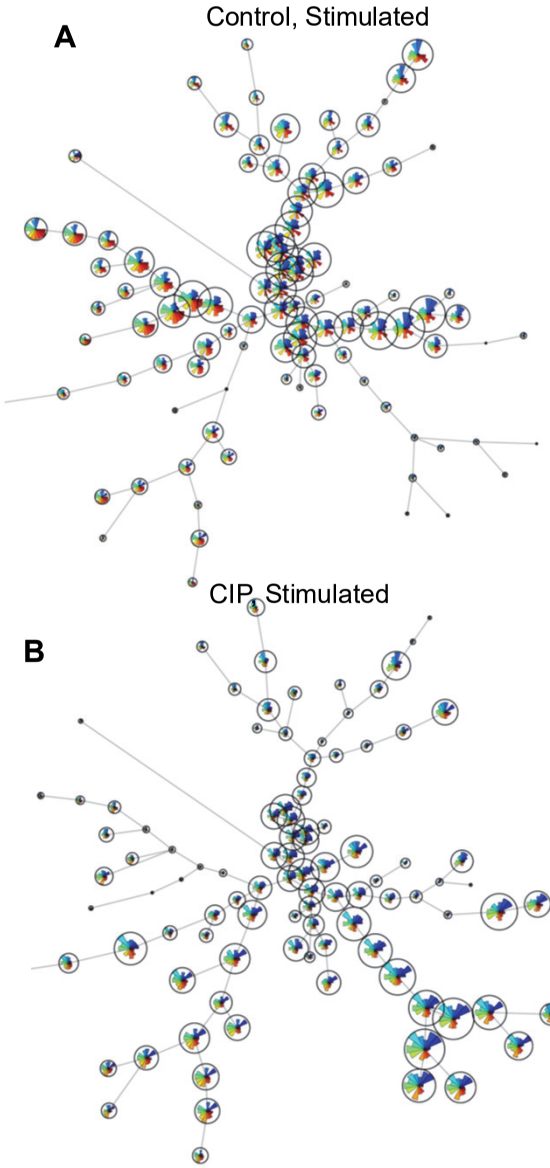
Supplemental Figure 4: Cluster maps showing distribution of T cell subpopulations in A) stimulated controls, B) stimulated controls. Within each cluster map, larger cell populations distinct to that particular condition are highlighted (square boxes). C) Legend showing fluorophore color coding for the nodes shown in cluster maps.

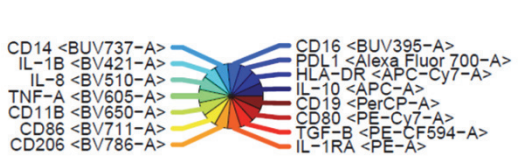


Supplemental Figure 5: Cluster maps of $CD3^{-}$ cells (i.e. myeloid/B cells) showing locations of $CD14^{hi}$, $CD16^{hi}$ and $CD19^{hi}$ subpopulation

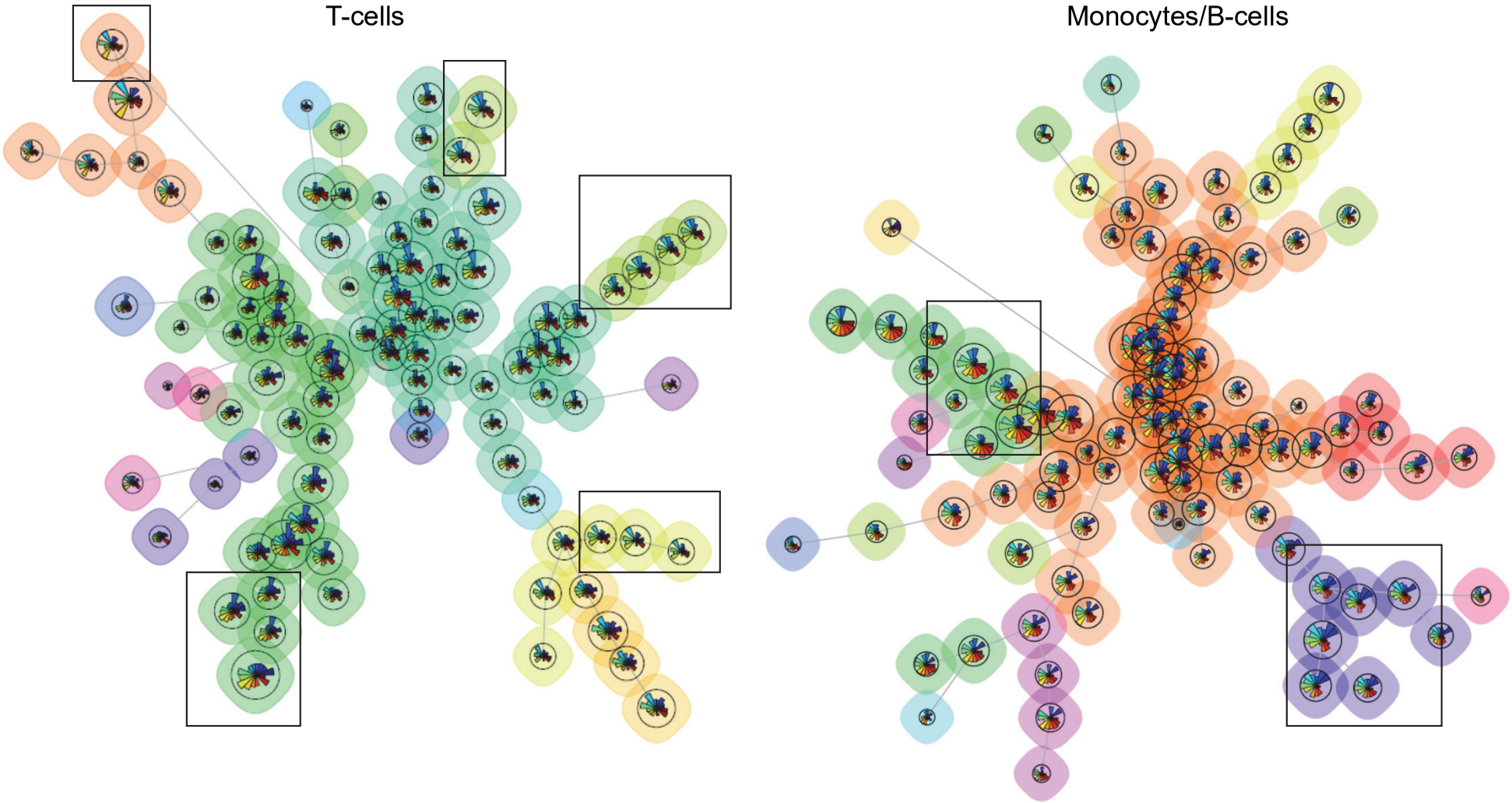


Supplemental Figure 6: A) Gating strategy for CD11b^{+} IL-1β^{+} cells. B) Histograms (normalized for event number) showing shift in TNF-a MFI in CIP compared to controls. C) Scatter plot showing TNF-alpha MFI in control and CIP * $p < 0.05$





Supplementary Figure 7: Unsupervised clustering of non-T cells (singlet, live, $CD3^-$) in BALF samples of patients without (Control) and with CIP. Cluster maps showing distribution of myeloid subpopulations in A) stimulated controls and B) stimulated CIP samples. C) Legend showing fluorophore color coding for the nodes shown in cluster maps.



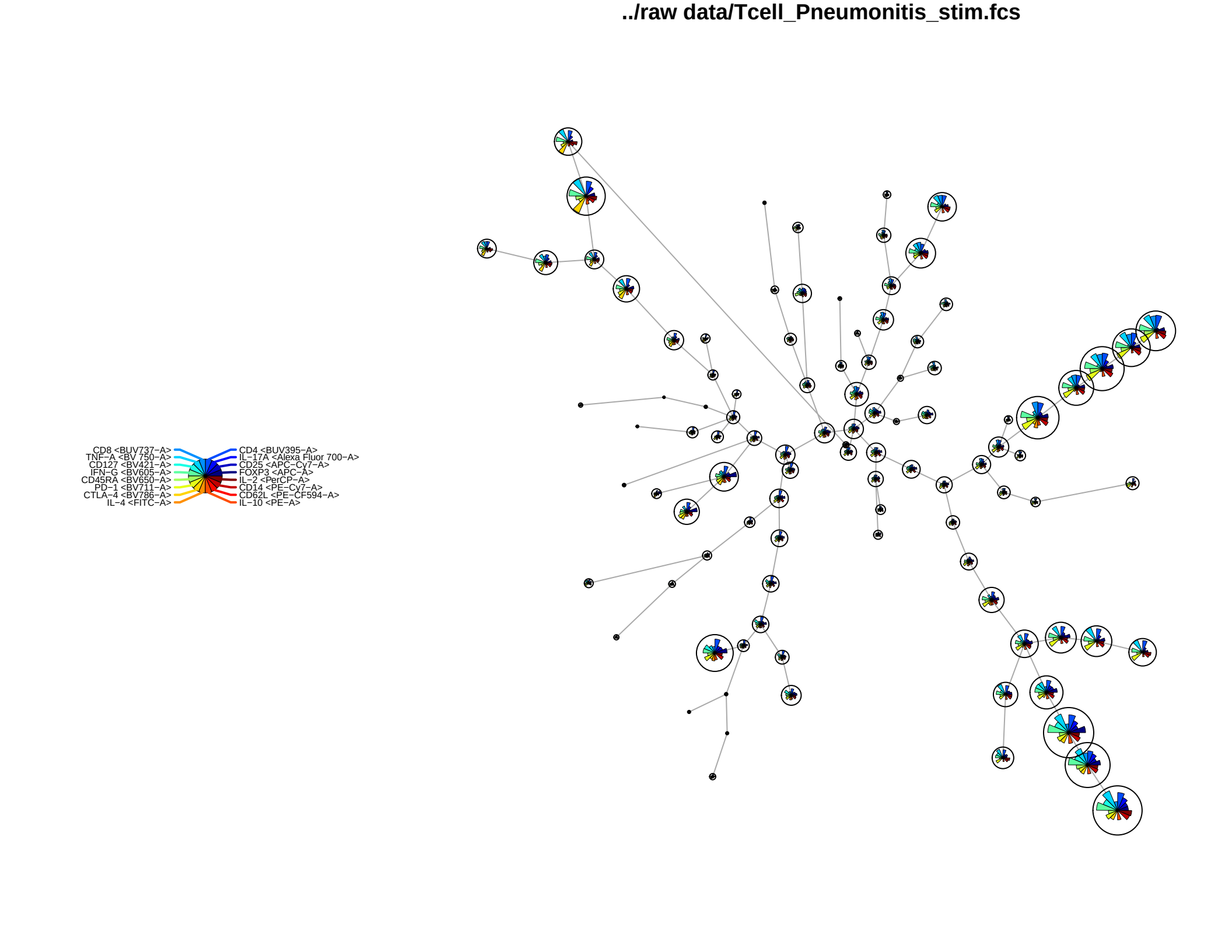
Supplemental Figure 8: Meta-clustering results for T-cells and Monocytes/B-cells. Following construction of a self-organizing map, an additional meta-clustering step is performed to group clusters. This is analogous to auto-gating. Squares represent distinct populations that were identified in the differential cluster map as being increased in either CIP or controls.

	Control		CIP		
	Mean	Std Dev	Mean	Std Dev	p value
IFNg	0.779667	1.45745	0.53115	1.361542	0.732284
IL10	0.081659	0.141755	0.014875	0.023957	0.302224
IL12	0.023937	0.031955	0.010554	0.02079	0.379205
IL13	0.111413	0.120524	0.013468	0.016766	0.103335
IL2	0.04545	0.070266	0.010836	0.012856	0.283282
IL4	0.009421	0.012905	0.004704	0.009524	0.447625
IL6	4.734932	5.4412	4.803961	10.45333	0.985147
GMCSF	0.030448	0.037562	0.032635	0.027533	0.901752
IL15	0.025567	0.019269	0.023409	0.024091	0.837823
IL16	10.15608	8.696985	5.176462	3.388175	0.226292
IL17A	0.154286	0.247953	0.69987	2.422319	0.436923
IL1a	0.413182	0.358688	0.068019	0.050755	0.064926
IL5	0.035908	0.035235	0.019017	0.020387	0.312742
IL7	0.09718	0.099613	0.075487	0.142147	0.707571
TNFb	0.000354	0.000868	0.000389	0.000976	0.938421
VEGF	13.20127	15.48359	3.388968	3.940829	0.183383
Eotaxin	0.717829	0.794458	1.000721	0.906459	0.504702
Eotaxin3	0.470828	0.760339	0.071615	0.091669	0.255427
MCP1	11.08744	17.97362	10.62813	9.071708	0.954644
MCP4	0.965623	1.426214	1.308574	1.510616	0.642507
MDC	0.448395	0.568259	1.706238	2.843228	0.148365
MIP1a	1.227391	0.635018	0.705749	0.628844	0.126851
MIP1b	2.02809	2.650103	1.735027	1.889067	0.813968
IL21	0.013214	0.023716	0.005838	0.01519	0.507478
IL22	0.053094	0.104954	0.075529	0.250447	0.786715
IL23	0.001636	0.004008	0.003126	0.007733	0.588297
IL27	0.493637	0.811066	0.837556	1.099269	0.458316
IL31	0.000701	0.000767	0.000555	0.00083	0.713563
CRP	5481.684	7654.096	12878.16	32920.19	0.455709
ICAM1	1142.413	1012.285	2074.535	2097.199	0.208967
SAA	2203.772	1688.405	11616.9	30960.36	0.295707
VCAM2	234.1811	214.7726	449.9151	435.3729	0.166676
bFGF	0.865423	1.141556	0.114848	0.161199	0.168742
VEGFR1	4.979985	3.78473	1.582051	1.899452	0.081097
PIGF	0.169554	0.198296	0.075905	0.116013	0.319899
TIE2	0.288373	0.706367	0.98758	1.522882	0.189427
VEGF.1	29.33105	36.61045	7.214126	9.081488	0.201249
VEGFD	1.858516	1.982102	2.825724	2.570295	0.078471

Supplementary Table 5: Mean, Std. dev and results of a two-tailed non-parametric t-test (p value) for cytokines not show in Figure 5

Supplemental Data

1. High Resolution Cluster Maps
2. Clustering code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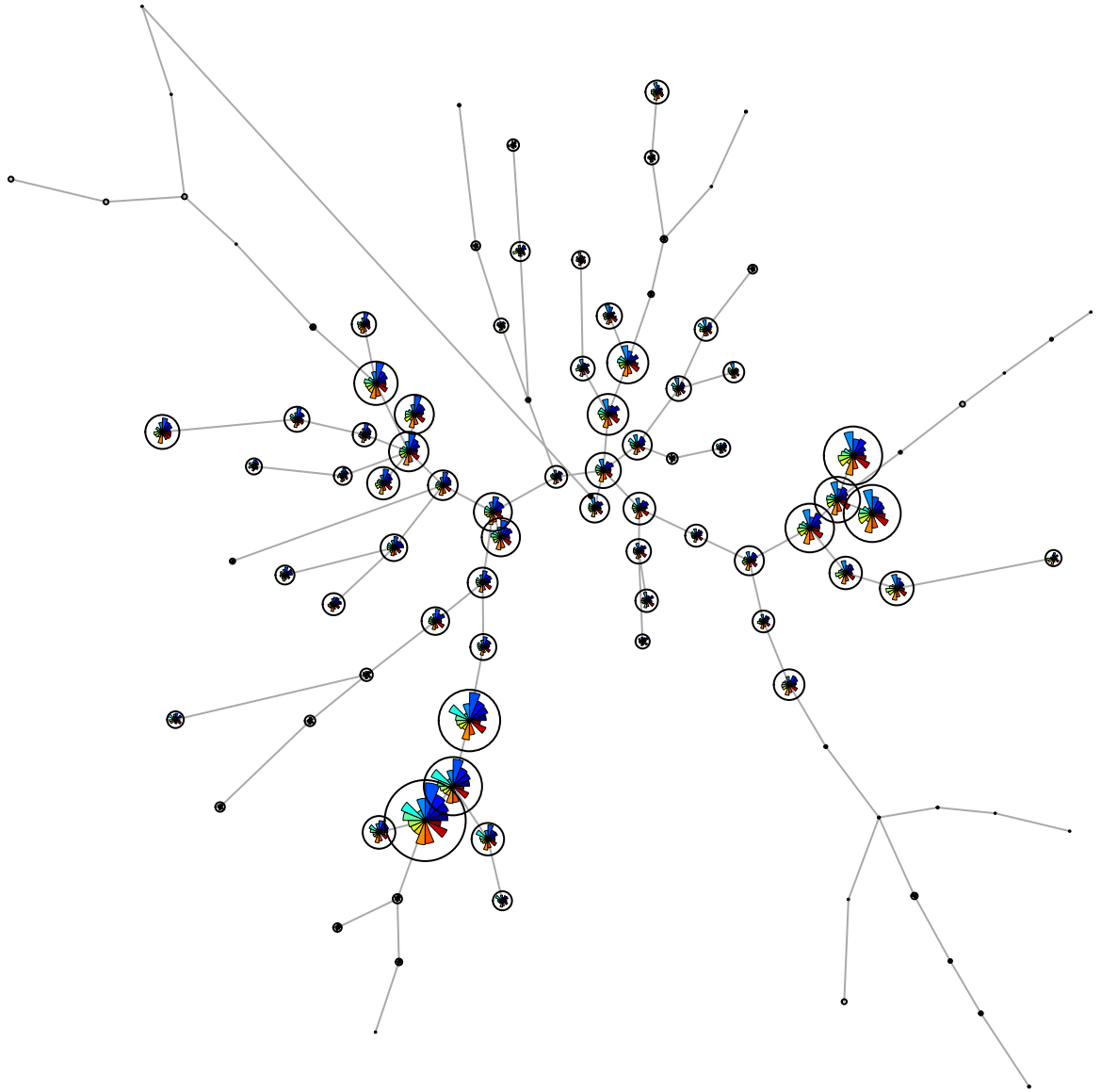

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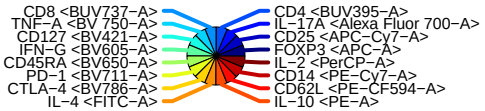

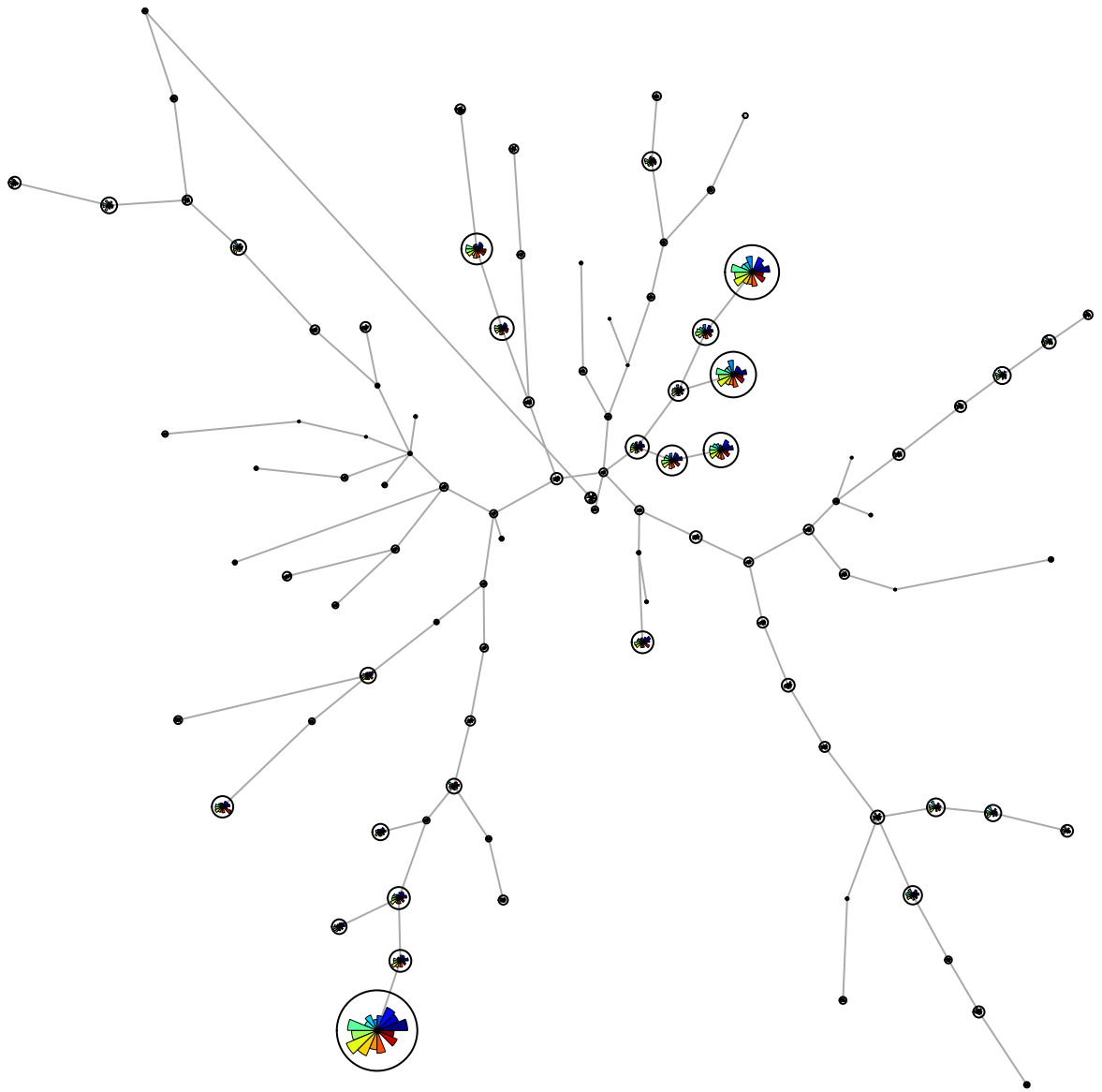

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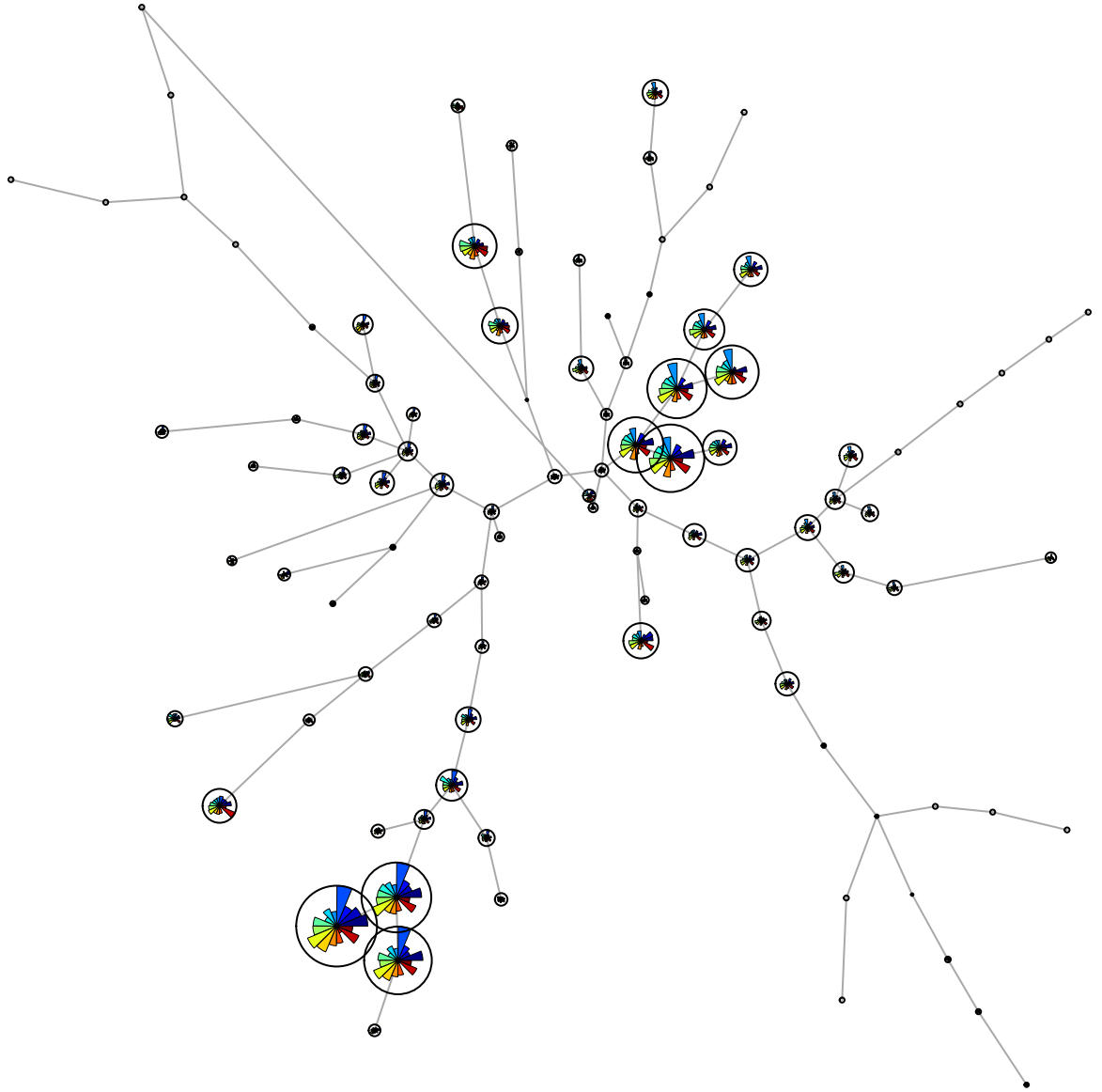

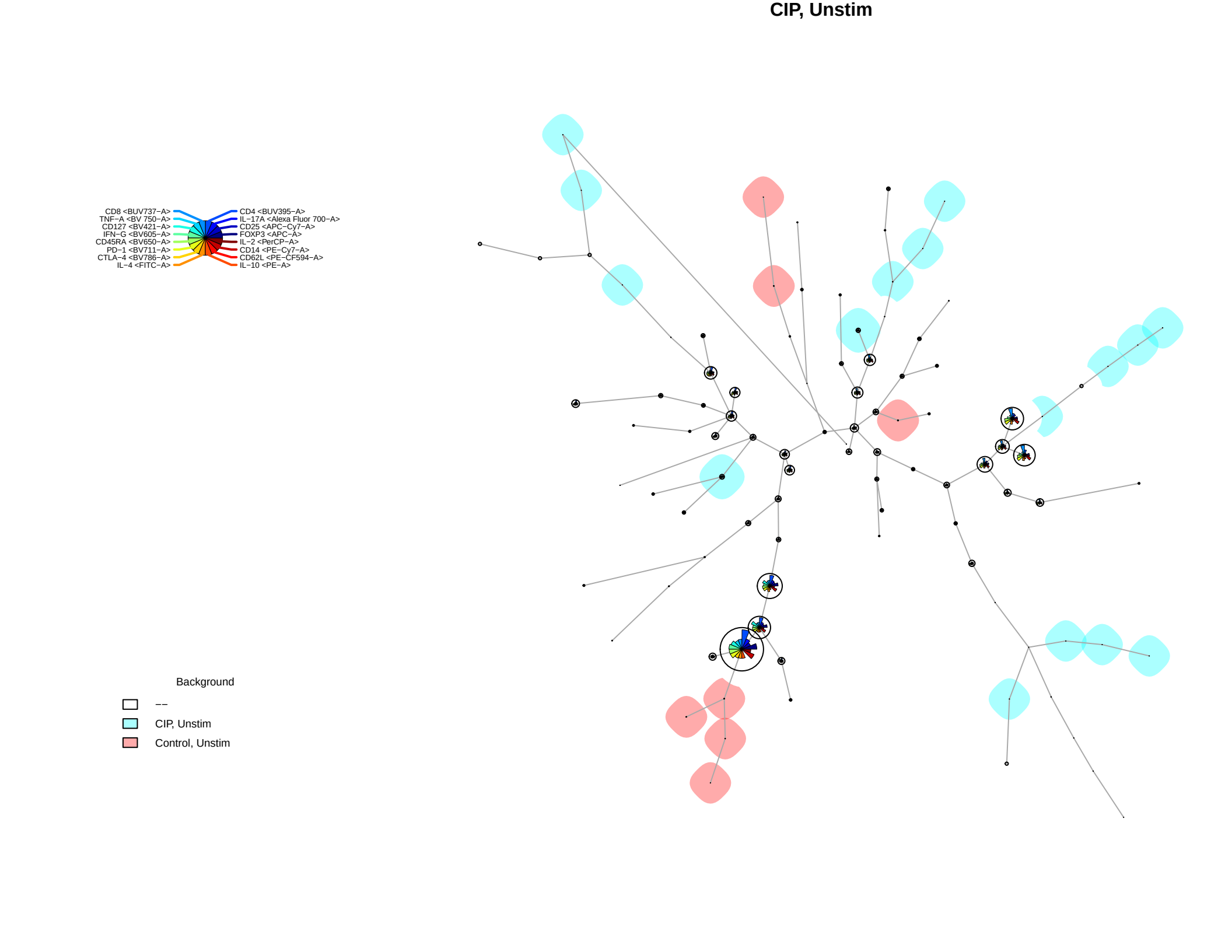
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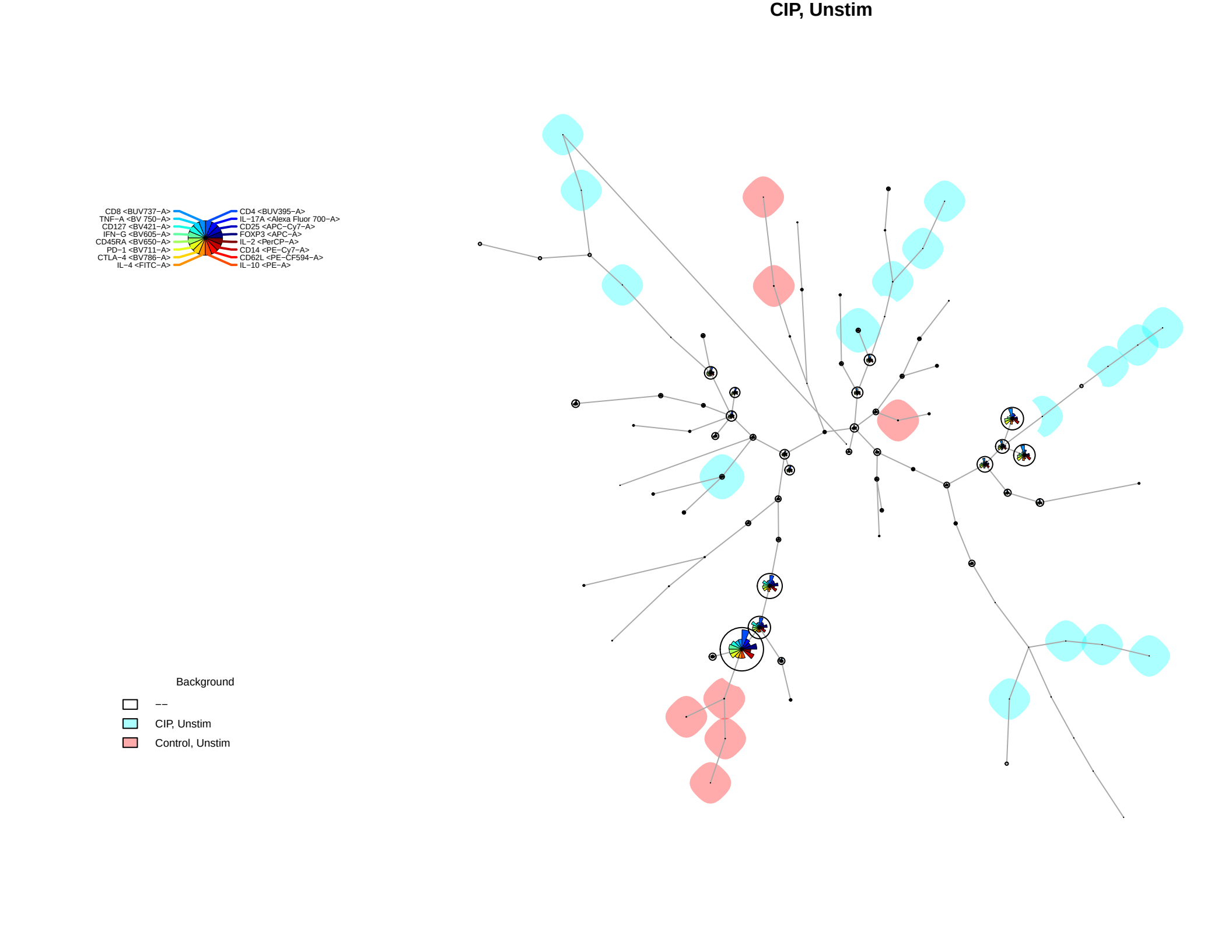
CIP, Unstim
Control, Unstim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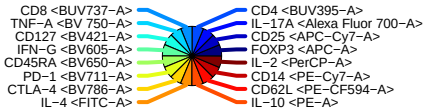
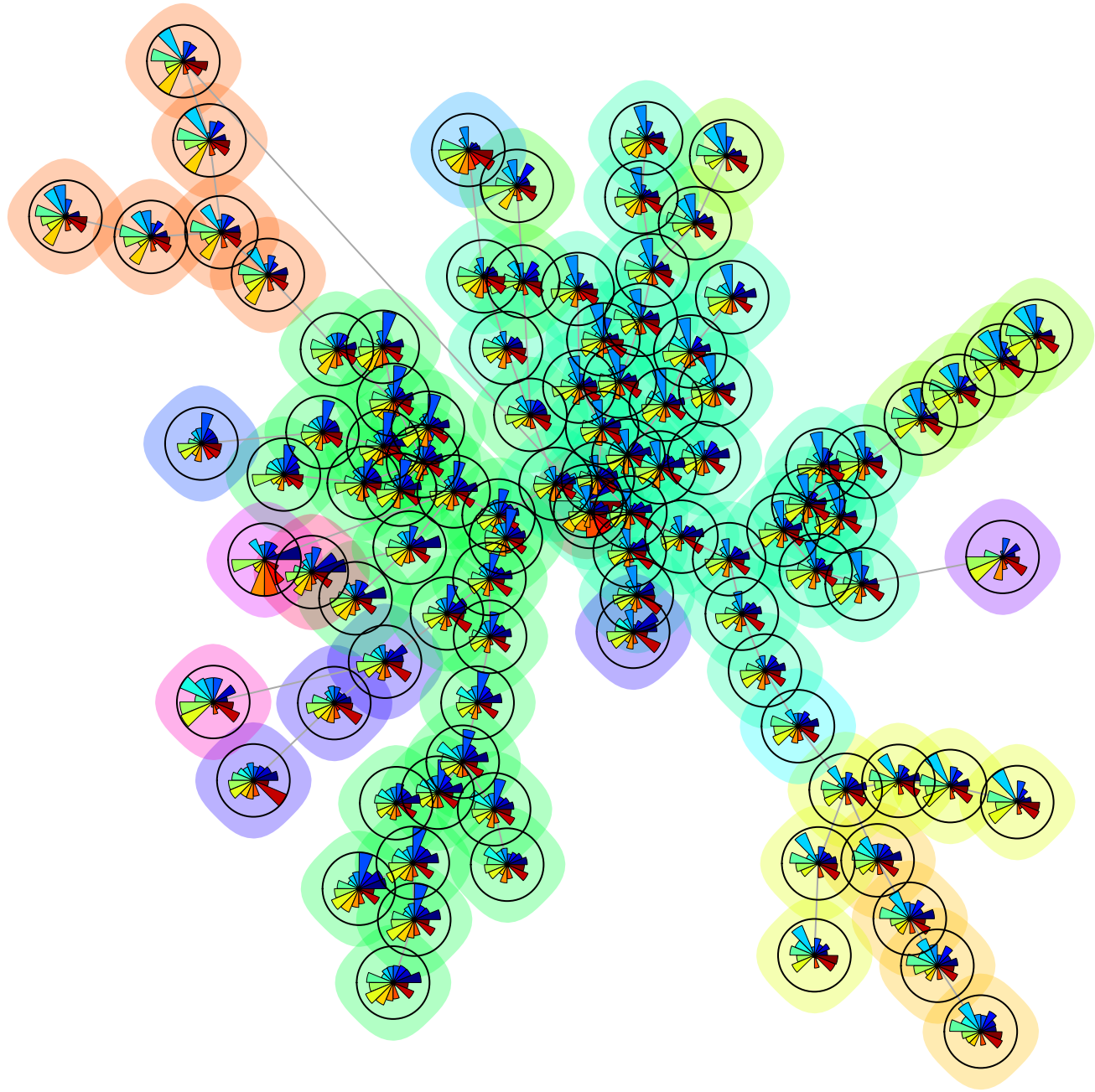
Control, Unstim





Background

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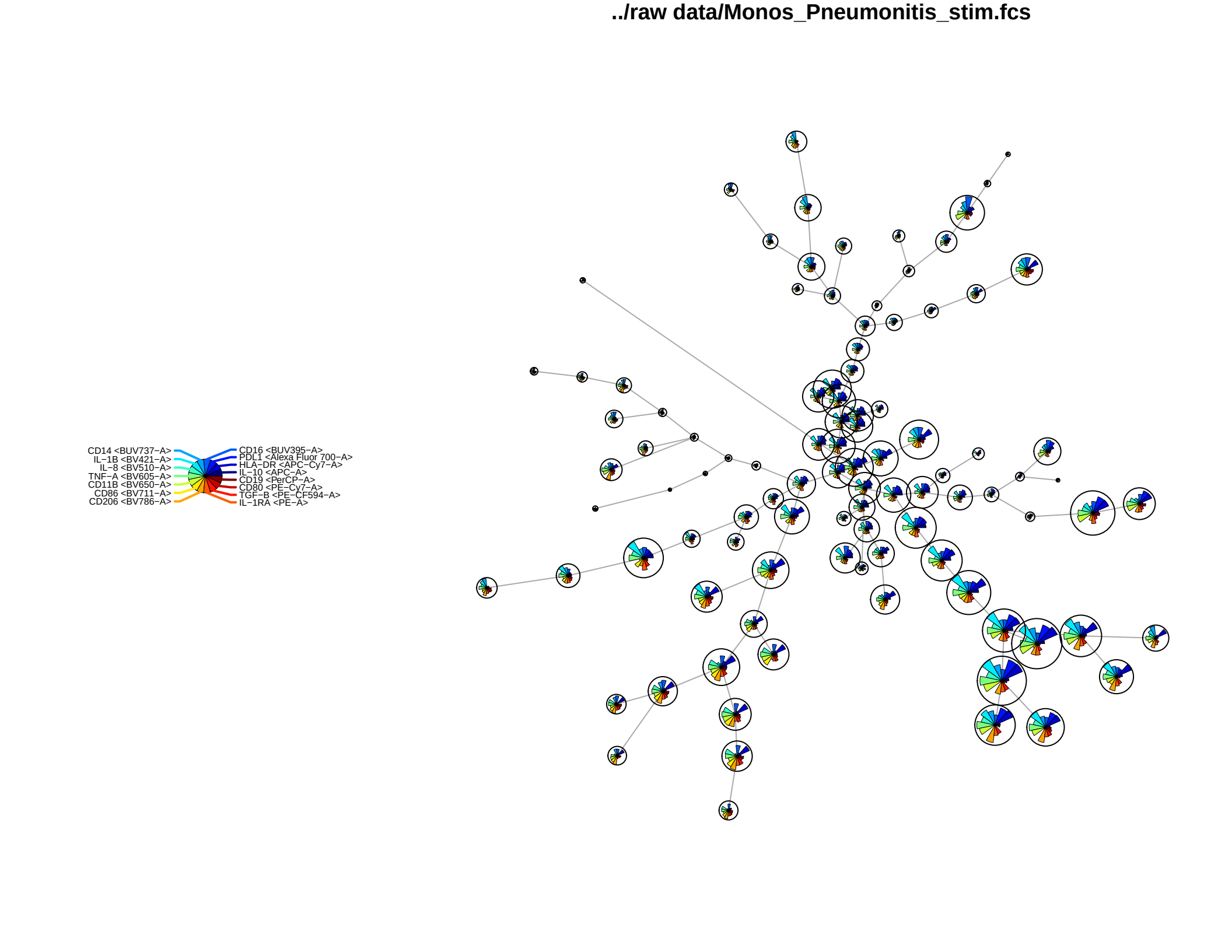
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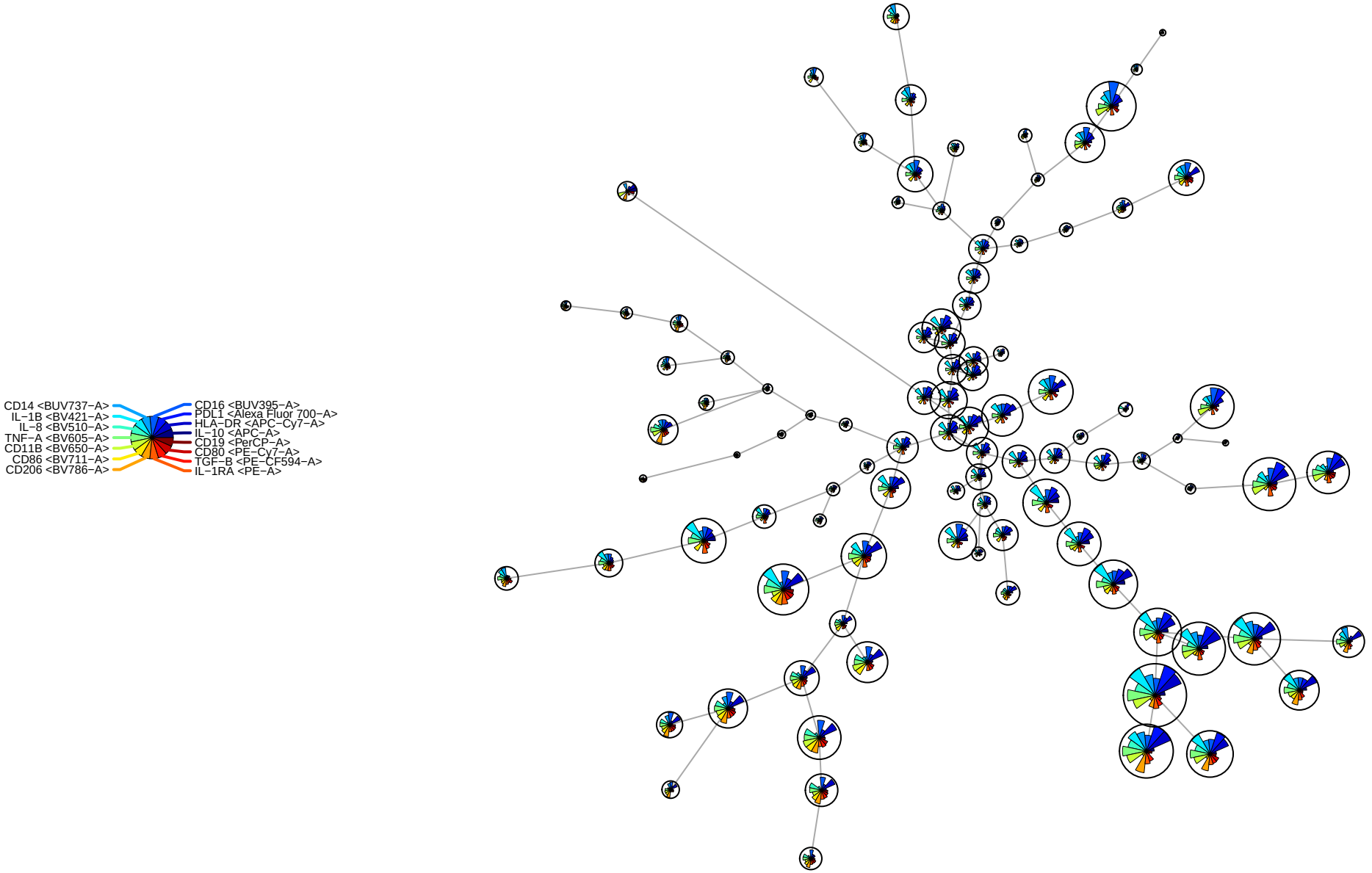
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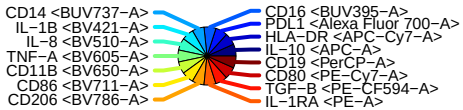

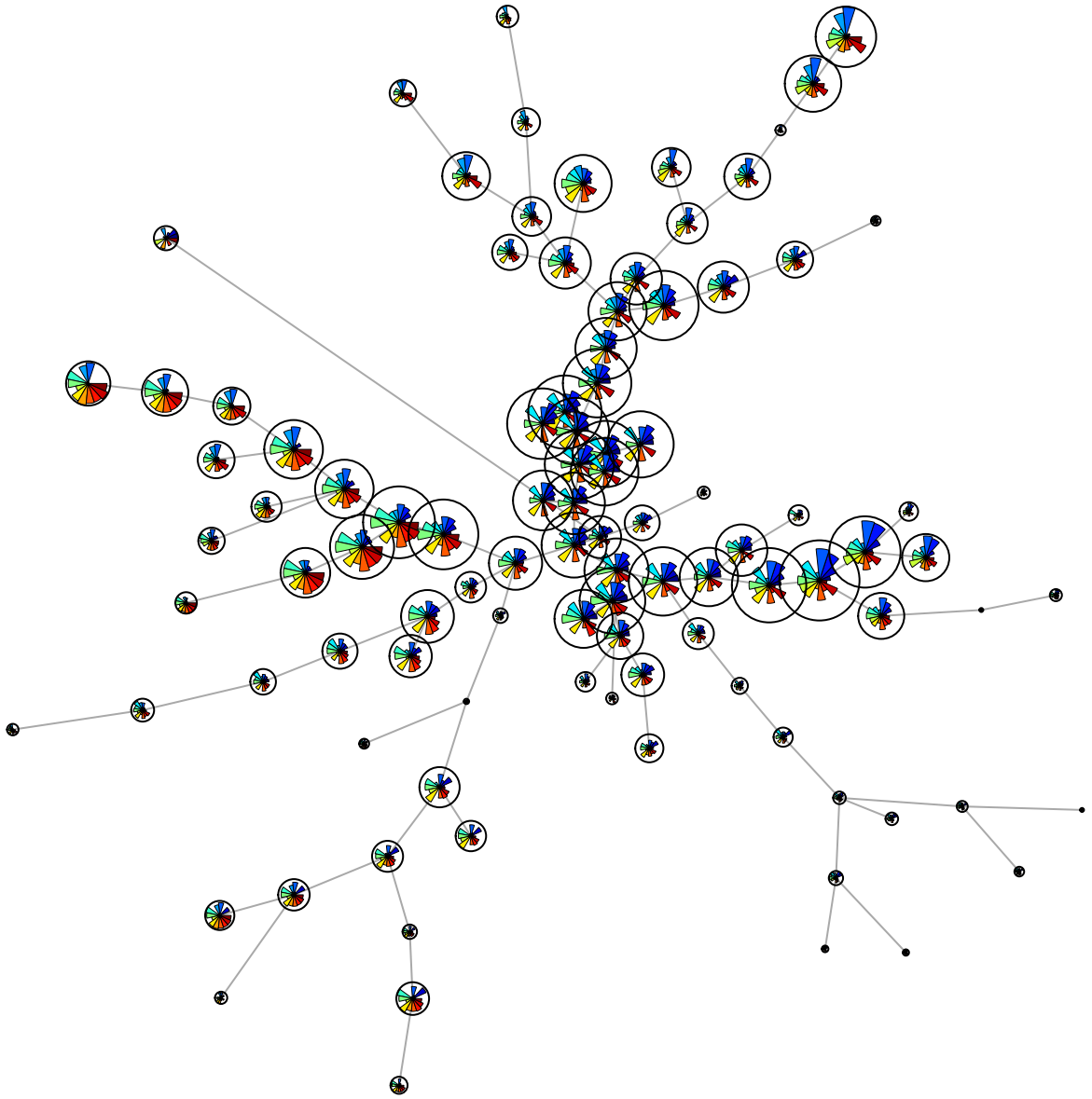

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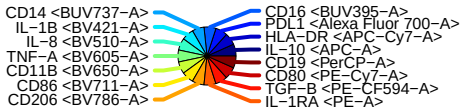

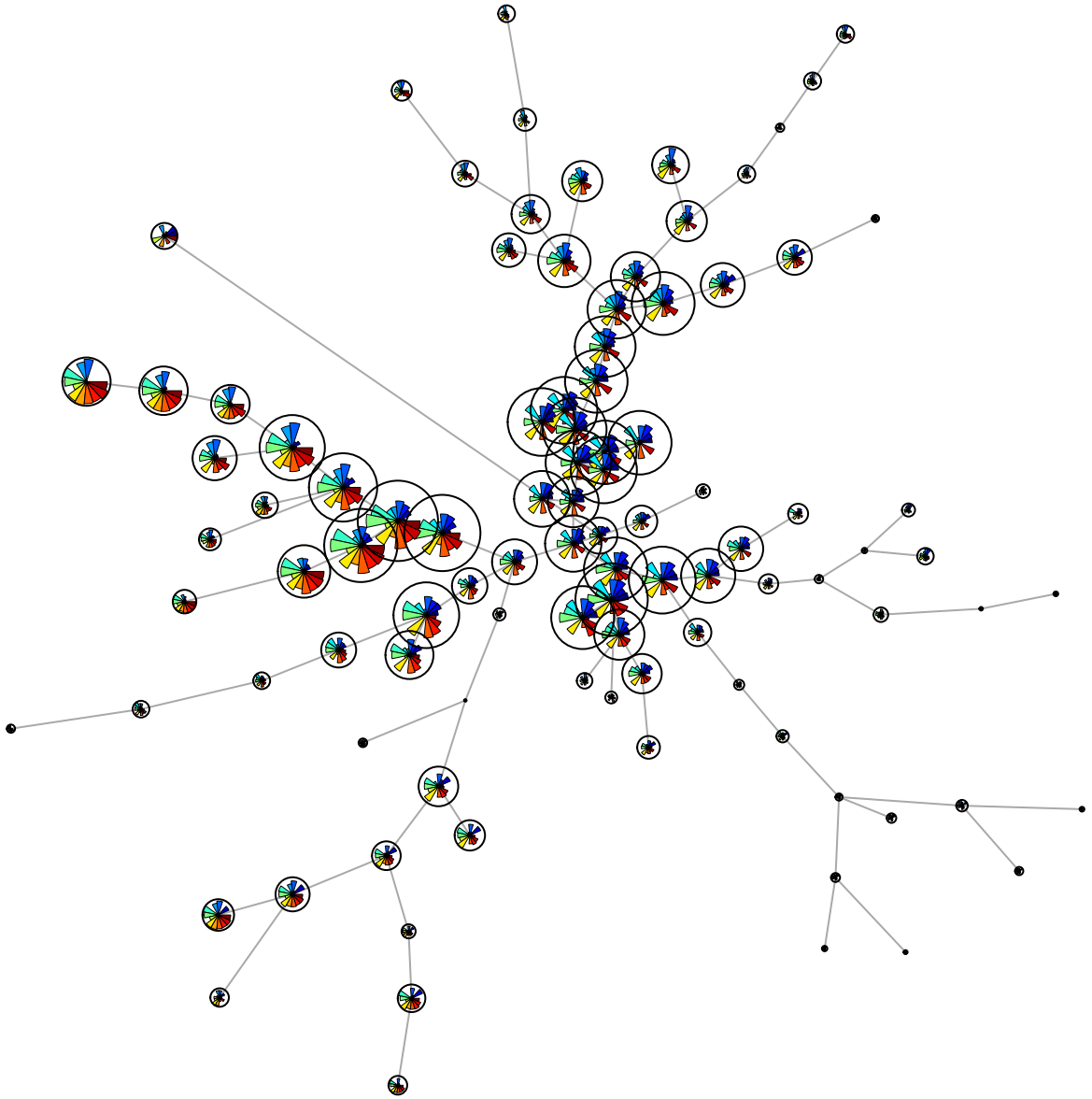

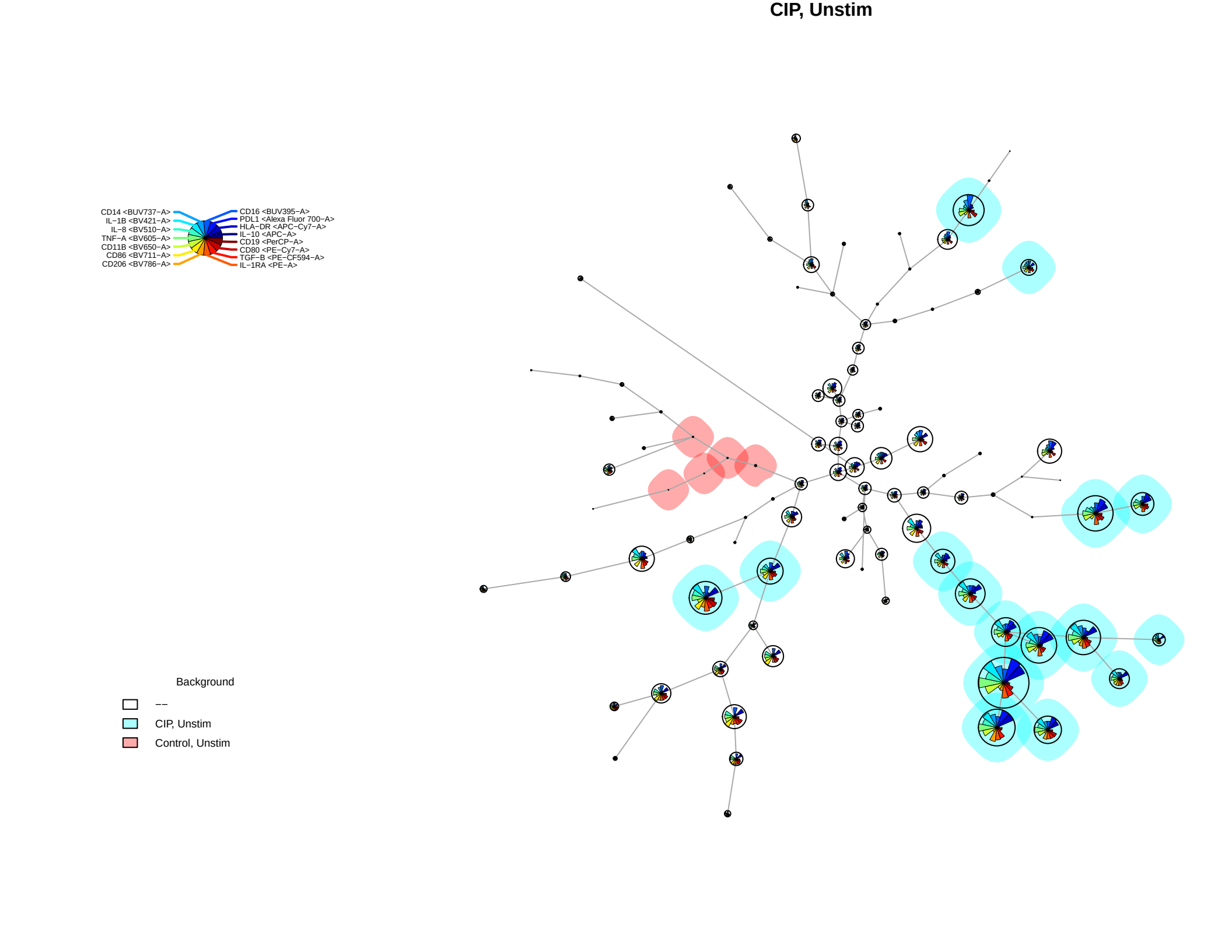
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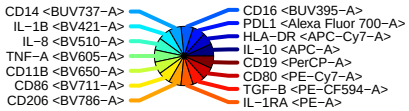
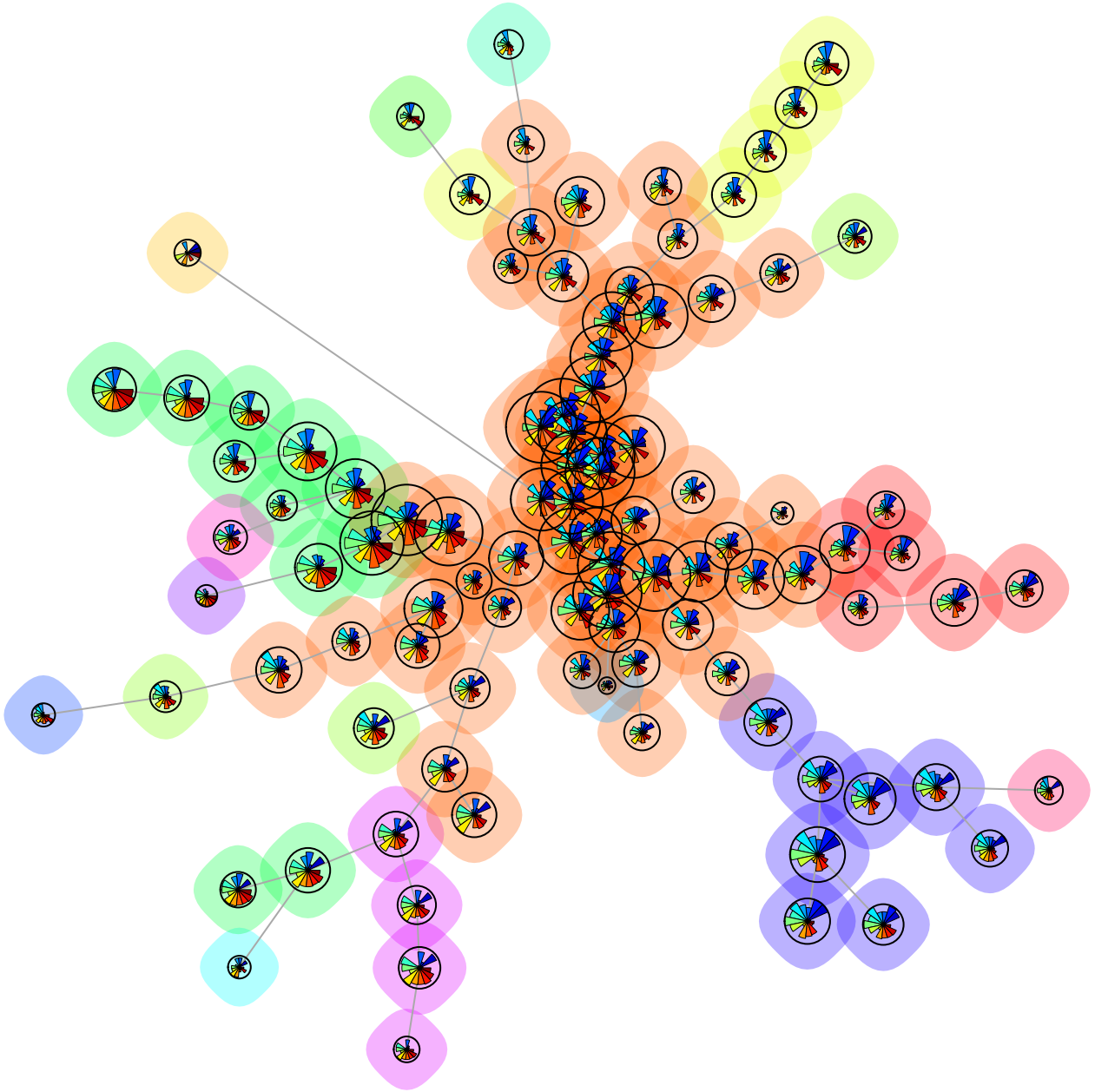
Background
−−
CIP, Unstim
Control, Unstim





Background

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CIP Flow - Unsupervised Analysis-With Stim samples

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T-cell Analysis

We import a legend that connects our sample IDs to their clinical data (pneumonitis status, etc). We then find the fcs files based on sample_id. Next, we aggregate all the fcs files by condition (CIP Y/N).We have already gated on live/dead and CD3+ (in FlowJo) before running these analyses (hence we're using fcs files from the cd3poslive folder).We have four groups: Control, Unstim; Control, Stim; CIP, Unstim; CIP, Stim. All are concatenated together to create the self organizing map (SOM).

```
knitr::opts_chunk$set(echo = TRUE)
library("FlowSOM")
library("flowCore")
library("dplyr")
sample_key <- read.csv("../raw data/sample.legends/122018_legend2.csv")
Controls <- sample_key[sample_key$pneumonitis==0 & is.na(sample_key$exclude)
& is.na(sample_key$pbmc),]
Pneumonitis <- sample_key[sample_key$pneumonitis==1 &
is.na(sample_key$exclude) & is.na(sample_key$pbmc),]

Controls$sample_id <- as.factor(Controls$sample_id)
Pneumonitis$sample_id <- as.factor(Pneumonitis$sample_id)

# we need to first gate on live/dead and cd3+ so that we're only dealing with
lymphocytes.

filenames <- as.data.frame(list.files("../raw data/HUMAN CIP RAW
FLOW/cd3poslive/", recursive=TRUE))

colnames(filenames) <- c("file")
# some processing of the filename to get out  the sample_id
filenames$sample_id <- (strsplit(as.character(filenames$file), "[:_:]"))
filenames$sample_id <- lapply(filenames$sample_id, function(x) return(x[3]))
filenames$stim <- grepl("PMA", filenames$sample_id)
filenames$sample_id <- strsplit(as.character(filenames$sample_id), " ")
filenames$sample_id <- lapply(filenames$sample_id, function(x) return(x[1]))

filenames$sample_id <- as.factor(as.character(filenames$sample_id))
Controls <- left_join(Controls, filenames, by=c("sample_id"))
Pneumonitis <- left_join(Pneumonitis, filenames, by=c("sample_id"))
```

```
# this connects sample_ID and file name
Controls$file <- paste0("../raw data/HUMAN CIP RAW FLOW/cd3poslive/",
as.character(Controls$file))
Pneumonitis$file <- paste0("../raw data/HUMAN CIP RAW FLOW/cd3poslive/",
as.character(Pneumonitis$file))

# Aggregating controls and CIPs, and for the master file, all samples
AggregateFlowFrames(as.character(Controls$file), cTotal=100000,
writeOutput=TRUE, outputFile="../raw data/Tcell_controls.fcs")
AggregateFlowFrames(as.character(Controls[Controls$stim==FALSE,]$file),
cTotal=100000, writeOutput=TRUE, outputFile="../raw
data/Tcell_controls_unstim.fcs")
AggregateFlowFrames(as.character(Controls[Controls$stim==TRUE,]$file),
cTotal=100000, writeOutput=TRUE, outputFile="../raw
data/Tcell_controls_stim.fcs")

AggregateFlowFrames(as.character(Pneumonitis$file), cTotal=100000,
writeOutput=TRUE, outputFile="../raw data/Tcell_Pneumonitis.fcs")
AggregateFlowFrames(as.character(Pneumonitis[Pneumonitis$stim==FALSE,]$file),
cTotal=100000, writeOutput=TRUE, outputFile="../raw
data/Tcell_Pneumonitis_unstim.fcs")
AggregateFlowFrames(as.character(Pneumonitis[Pneumonitis$stim==TRUE,]$file),
cTotal=100000, writeOutput=TRUE, outputFile="../raw
data/Tcell_Pneumonitis_stim.fcs")

# create master fcs
AggregateFlowFrames(c(as.character(Controls$file),
as.character(Pneumonitis$file)), cTotal=100000, writeOutput=TRUE,
outputFile="../raw data/Tcell_Master_withStim.fcs")

# read a raw FCS file to understand its structure. This was done so we can
get some sense of the flowCore data structure and figure out which lasers
corresponded to which fluorophores
ff_B1 <- read.FCS("../raw data/HUMAN CIP RAW FLOW/cd3poslive/export_T cell_1
MED_009_CD3.fcs", transformation=FALSE)

# we need to pick out the right lasers. Looking at summary(ff_B1) gives us
the structure. We exclude live/dead and cd3 sincce already gated on those.
lasers  <- c(7:13,15:19,21:24)
lasers_monos <- c(7:11,13:18, 21:24)

# this is the actual clustering command. This creates a structure with 2
components. fSOM_Master_withStim[[1]] is a flowSOM structure. [[2]] is the
meta-clustering results.

fSOM_Master_withStim <- FlowSOM("../raw data/Tcell_Master_withStim.fcs",
compensate=TRUE, transform=TRUE, toTransform=lasers, scale=TRUE,
colsToUse=lasers, nClus=16, seed=31)
```

```
fSOM_Master_withStim[[1]] <- BuildMST(fSOM_Master_withStim[[1]], tSNE=TRUE)

# meta-clustered results
PlotStars(fSOM_Master_withStim$FlowSOM, backgroundValues =
as.factor(fSOM_Master_withStim$metaclustering))

# group comparisons
groupRes <- CountGroups(fSOM_Master_withStim[[1]], groups=list("Control,
Unstim"=c("../raw data/Tcell_controls_unstim.fcs"), "Control, Stim"=c("../raw
data/Tcell_controls_stim.fcs")))

groupRes_CIP <- CountGroups(fSOM_Master_withStim[[1]], groups=list("CIP,
Unstim"=c("../raw data/Tcell_Pneumonitis_unstim.fcs"), "CIP, Stim"=c("../raw
data/Tcell_Pneumonitis_stim.fcs")))

groupRes_ControlvCIP <- CountGroups(fSOM_Master_withStim[[1]],
groups=list("Control, Unstim"=c("../raw data/Tcell_controls_unstim.fcs"),
"CIP, Unstim"=c("../raw data/Tcell_Pneumonitis_unstim.fcs")))

groupRes_ControlvCIPStim <- CountGroups(fSOM_Master_withStim[[1]],
groups=list("Control, Stim"=c("../raw data/Tcell_controls_stim.fcs"), "CIP,
Stim"=c("../raw data/Tcell_Pneumonitis_stim.fcs")))

# set equal node size for these graphs?
fSOM_Master_withStim[[1]] <- UpdateNodeSize(fSOM_Master_withStim[[1]])
PlotStars(fSOM_Master_withStim[[1]])

# plot differential cluster maps
PlotGroups(fSOM_Master_withStim[[1]], groupRes, tresh = 0.95)
PlotGroups(fSOM_Master_withStim[[1]], groupRes_CIP, tresh = 0.95)
PlotGroups(fSOM_Master_withStim[[1]], groupRes_ControlvCIP, tresh = 0.95,
view="grid")
PlotGroups(fSOM_Master_withStim[[1]], groupRes_ControlvCIPStim, tresh = 0.95)



# Where are the CD4+ cells?
PlotMarker(fSOM_Master_withStim[[1]], "BUV395-A")

# where are the CD8+ cells?
PlotMarker(fSOM_Master_withStim[[1]], "BUV737-A")

# where are the FoxP3 cells?
PlotMarker(fSOM_Master_withStim[[1]], "APC-A")

# where are the CD62L+ cells?
PlotMarker(fSOM_Master_withStim[[1]], "PE-CF594-A")

# where are the IL-2+ cells?
PlotMarker(fSOM_Master[[1]], "PerCP-A")
```

```
# where are the IFN-g+ cells?
PlotMarker(fSOM_Master[[1]], "BV605-A")

# where are the cd-14+ cells?
PlotMarker(fSOM_Master[[1]], "PE-Cy7-A")

# where are cd45RA cells
PlotMarker(fSOM_Master[[1]], "BV650-A")

# grid view
PlotStars(fSOM_Master[[1]], view="grid")

# plot nodes with numbers
PlotNumbers(UpdateNodeSize(fSOM_Master_withStim[[1]],reset=TRUE),
nodeSize=15)
PlotNumbers(UpdateNodeSize(fSOM_Master_withStim[[1]],reset=TRUE),
nodeSize=15, view="grid")

# 2d plots for PD1hi to lo switch
PlotClusters2D(fSOM_Master_withStim[[1]], "BV711-A", "BV786-A", c(52,71,61))
PlotClusters2D(fSOM_Master_withStim[[1]], "BV711-A", "BV786-A", c(75,91,92))


#2d for TNFa high cd8+
PlotClusters2D(fSOM_Master_withStim[[1]], "BUV737-A", "BV 750-A", c(8,9,18))

# indeterminate CD4/8 and TNF-a
PlotClusters2D(fSOM_Master_withStim[[1]], "BUV395-A", "BV 750-A", c(6,7,17))
PlotClusters2D(fSOM_Master_withStim[[1]], "BUV737-A", "BV 750-A", c(6,7,17))


# loss of CD4 with stimulation
PlotClusters2D(fSOM_Master_withStim[[1]], "BUV395-A", "APC-A", c(71))
PlotClusters2D(fSOM_Master_withStim[[1]], "BUV395-A", "APC-A", c(61,81,52))


# tcm in cip
PlotClusters2D(fSOM_Master_withStim[[1]], "BV650-A", "PE-CF594-A", c(11))
```

Monocyte Analysis

This is a repeat of the T-cell analysis but using singlet/live+/CD3- (instead of CD3+) cells as input.

```
filenames_monos <- as.data.frame(list.files("../raw data/HUMAN CIP RAW
FLOW/cd3negative/"))
colnames(filenames_monos) <- c("file")
```

```
filenames_monos$sample_id <- (strsplit(as.character(filenames_monos$file),
"[:_:]"))
filenames_monos$sample_id <- lapply(filenames_monos$sample_id, function(x)
return(x[3]))
filenames_monos$stim <- grepl("LPS", filenames_monos$sample_id)
filenames_monos$sample_id <-
strsplit(as.character(filenames_monos$sample_id), " ")
filenames_monos$sample_id <- lapply(filenames_monos$sample_id, function(x)
return(x[1]))

filenames_monos$sample_id <-
as.factor(as.character(filenames_monos$sample_id))

Controls_Monos <- sample_key[sample_key$pneumonitis==0 &
is.na(sample_key$exclude) & is.na(sample_key$pbmc),]
Pneumonitis_Monos <- sample_key[sample_key$pneumonitis==1 &
is.na(sample_key$exclude) & is.na(sample_key$pbmc),]



Controls_Monos <- left_join(Controls_Monos, filenames_monos,
by=c("sample_id"))
Pneumonitis_Monos <- left_join(Pneumonitis_Monos, filenames_monos,
by=c("sample_id"))

Controls_Monos$file <- paste0("../raw data/HUMAN CIP RAW FLOW/cd3negative/",
as.character(Controls_Monos$file))
Pneumonitis_Monos$file<- paste0("../raw data/HUMAN CIP RAW
FLOW/cd3negative/", as.character(Pneumonitis_Monos$file))

# concatenate fcs file by group
AggregateFlowFrames(as.character(Controls_Monos$file), cTotal=1000000,
writeOutput=TRUE, outputFile="../raw data/Monos_controls.fcs")
AggregateFlowFrames(as.character(Controls_Monos[Controls_Monos$stim==FALSE,
]$file), cTotal=1000000, writeOutput=TRUE, outputFile="../raw
data/Monos_controls_unstim.fcs")
AggregateFlowFrames(as.character(Controls_Monos[Controls_Monos$stim==TRUE,
]$file), cTotal=1000000, writeOutput=TRUE, outputFile="../raw
data/Monos_controls_stim.fcs")

AggregateFlowFrames(as.character(Pneumonitis_Monos$file), cTotal=1000000,
writeOutput=TRUE, outputFile="../raw data/Monos_Pneumonitis.fcs")
AggregateFlowFrames(as.character(Pneumonitis_Monos[Pneumonitis_Monos$stim==FA
LSE,]$file), cTotal=10000000, writeOutput=TRUE, outputFile="../raw
data/Monos_Pneumonitis_unstim.fcs")
AggregateFlowFrames(as.character(Pneumonitis_Monos[Pneumonitis_Monos$stim==TR
UE,]$file), cTotal=10000000, writeOutput=TRUE, outputFile="../raw
data/Monos_Pneumonitis_stim.fcs")

AggregateFlowFrames(c(as.character(Controls_Monos$file),
```

```
as.character(Pneumonitis_Monos$file)), cTotal=10000000, writeOutput=TRUE,
outputFile="../raw data/Monos_Master.fcs")

ff_B1_monos <- read.FCS("../raw data/HUMAN CIP RAW FLOW/monos/export_MONO_B1
MED_011_non T B cell.fcs", transformation=FALSE)

fSOM_Master_Monos <- FlowSOM("../raw data/Monos_Master.fcs", compensate=TRUE,
transform=TRUE, toTransform=lasers_monos, scale=TRUE, colsToUse=lasers_monos,
nClus=16, seed=42)
fSOM_Master_Monos[[1]] <- BuildMST(fSOM_Master_Monos[[1]], tSNE=TRUE)

# meta-clustered results
PlotStars(fSOM_Master_Monos$FlowSOM, backgroundValues =
as.factor(fSOM_Master_Monos$metaclustering))

# group comparisons
groupRes_Monos_Control <- CountGroups(fSOM_Master_Monos[[1]],
groups=list("Controls"=c("../raw data/Monos_controls_unstim.fcs"),
"CIP"=c("../raw data/Monos_controls_stim.fcs")))
groupRes_Monos_CIP <- CountGroups(fSOM_Master_Monos[[1]],
groups=list("Controls"=c("../raw data/Monos_Pneumonitis_unstim.fcs"),
"CIP"=c("../raw data/Monos_Pneumonitis_stim.fcs")))
groupRes_Monos_ControlvCIP_unstim <- CountGroups(fSOM_Master_Monos[[1]],
groups=list("Control, Unstim"=c("../raw data/Monos_controls_unstim.fcs"),
"CIP, Unstim"=c("../raw data/Monos_Pneumonitis_unstim.fcs")))
groupRes_Monos_ControlvCIP_stim <- CountGroups(fSOM_Master_Monos[[1]],
groups=list("Control, Stim"=c("../raw data/Monos_controls_stim.fcs"), "CIP,
Stim"=c("../raw data/Monos_Pneumonitis_stim.fcs")))


PlotGroups(fSOM_Master_Monos[[1]], groupRes_Monos_Control, tresh = 0.95)
PlotGroups(fSOM_Master_Monos[[1]], groupRes_Monos_CIP, tresh = 0.95)
PlotGroups(fSOM_Master_Monos[[1]], groupRes_Monos_ControlvCIP_unstim, tresh =
0.95)
PlotGroups(fSOM_Master_Monos[[1]], groupRes_Monos_ControlvCIP_stim, tresh =
0.95)

# plot numbers
PlotNumbers(UpdateNodeSize(fSOM_Master_Monos[[1]],reset=TRUE), nodeSize=15)


# where are the CD14 cells?
PlotMarker(fSOM_Master_Monos[[1]], "BUV737-A")
# Where are the CD16 cells?
PlotMarker(fSOM_Master_Monos[[1]], "BUV395-A")
PlotMarker(fSOM_Master_Monos[[1]], "PerCP-A")


#2d plots for the mono cluster
```

```
#IL-1B/CD11B
PlotClusters2D(fSOM_Master_Monos[[1]], "BV421-A", "BV650-A", c(88, 98, 99,
80,90,100,89))
PlotClusters2D(fSOM_Master_Monos[[1]], "BV421-A", "BV650-A", c(61,62,91,92))

#CD19/IL-1RA
PlotClusters2D(fSOM_Master_Monos[[1]], "PerCP-A", "PE-A", c(72,73,74,64))

#CD14/CD16
PlotClusters2D(fSOM_Master_Monos[[1]], "BUV737-A", "BUV395-A",
c(65,56,75,76))
PlotClusters2D(fSOM_Master_Monos[[1]], "BUV737-A", "BUV395-A",
c(61,62,91,92))

#CD14/IL-1b
PlotClusters2D(fSOM_Master_Monos[[1]], "BUV737-A", "BV421-A", c(65,56,75,76))
PlotClusters2D(fSOM_Master_Monos[[1]], "BUV737-A", "BV421-A", c(61,62,91,92))
#CD16/IL-1b
PlotClusters2D(fSOM_Master_Monos[[1]], "BUV395-A", "BV421-A", c(65,56,75,76))
PlotClusters2D(fSOM_Master_Monos[[1]], "BUV395-A", "BV421-A", c(61,62,91,92))

# cd19/PD-L1
PlotClusters2D(fSOM_Master_Monos[[1]], "PerCP-A", "BV650-A", c(98,89,99,100))
PlotClusters2D(fSOM_Master_Monos[[1]], "PerCP-A", "BV650-A", c(80,69,60,70))
```

R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see http://rmarkdown.rstudio.com.

When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

```
summary(cars)
```

```
##      speed           dist
##  Min.   : 4.0   Min.   :  2.00
##  1st Qu.:12.0   1st Qu.: 26.00
##  Median :15.0   Median : 36.00
##  Mean   :15.4   Mean   : 42.98
##  3rd Qu.:19.0   3rd Qu.: 56.00
##  Max.   :25.0   Max.   :120.00
```

Including Plots

You can also embed plots, for example: